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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of	Examiner: Not yet assigned
Kindsvogel *et al.*	
Serial No.: Not yet assigned	Art Unit: Not yet assigned
Filed: Herewith	PRELIMINARY AMENDMENT
For: IMMUNE MEDIATORS AND RELATED METHODS	

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above-identified application as follows.

IN THE SPECIFICATION

On page 1, lines 7-12, please amend the application as follows:

The present application is a continuation of U.S. Serial No 09/261,811, filed March 3, 1999, and U.S. Serial No 08/657,581, filed June 7, 1996, and is a continuation-in-part of U.S. Serial No. 08/480,002, filed June 7, 1995, U.S. Serial No. 08/483,241, filed June 7, 1995 and U.S. Serial No. 08/482,133, filed June 7, 1995, and claims the benefit of U.S. Provisional Application No. 60/005,964, filed October 27, 1995, the disclosures of which are herein each incorporated by reference in their entirety.

IN THE CLAIMS

Please cancel claims 1-26 without prejudice to subsequent revival.

Please add new claims 27-49 as follows.

27. (new) A soluble, fused major histocompatibility complex (MHC) class II heterodimer, which forms a peptide binding groove that associates with an antigenic peptide, the MHC class II heterodimer comprising the following elements covalently linked in sequence:

a first polypeptide segment consisting essentially of a β1 domain of an MHC class II chain;

a first peptide linker segment; and

a second polypeptide segment consisting essentially of an α1 domain of an MHC class II chain, wherein said β1 domain and said α1 domain form the peptide binding groove of the MHC class II heterodimer.

28. (new) The MHC class II heterodimer of claim 27, wherein the MHC class II heterodimer further comprises:

a third polypeptide segment comprising an antigenic peptide that associates with the peptide binding groove of the MHC class II heterodimer; and

a second peptide linker segment connecting the third and first polypeptide segments.

29. (new) The MHC class II heterodimer of claim 27, wherein the MHC class II β1 domain is from a human DR1β*1501 β1 domain.

30. (new) The MHC class II heterodimer of claim 27, wherein the MHC class II α 1 domain is from a human DRA*0101 α1 domain.

31. (new) The MHC class II heterodimer of claim 27, wherein the first peptide linker is about 5 to about 25 amino acids in length.

32. (new) The MHC class II heterodimer of claim 28, wherein the first and the second peptide linkers are about 5 to about 25 amino acids in length.

33. (new) The MHC class II heterodimer of claim 31 or 32, wherein the first peptide linker segment has the sequence GASAG (SEQ ID NO:29) or GGSGGS (SEQ ID NO:31).

34. (new) The MHC class II heterodimer of claim 32, wherein the second peptide linker segment has the sequence GASAG (SEQ ID NO:29) or GGSGGS (SEQ ID NO:31).

35. (new) The MHC class II heterodimer of claim 28, wherein the third polypeptide segment is antigenic peptide capable of stimulating a CD4+ helper T cell-mediated immune response.

36. (new) The MHC class II heterodimer of claim 35, wherein the third polypeptide segment is a peptide selected from the group consisting of SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:40, SEQ ID NO:39, and SEQ ID NO:33.

37. (new) A pharmaceutical composition comprising a soluble, fused MHC class II heterodimer of claim 27 in combination with a pharmaceutically acceptable carrier.

38. (new) A pharmaceutical composition comprising a soluble, fused MHC class II heterodimer:peptide complex of claim 28 in combination with a pharmaceutically acceptable carrier.

39. (new) An expression cassette encoding a soluble, fused major histocompatibility complex (MHC) class II heterodimer, which forms a peptide binding groove that associates with an antigenic peptide, the expression cassette comprising the following operably linked elements:

a transcription promoter;

a first nucleic acid segment encoding a first polypeptide segment consisting essentially of a β1 domain of an MHC class II chain;

a second nucleic acid segment encoding a second polypeptide segment consisting essentially of an α1 domain of an MHC class II chain; and

a first linker segment encoding a first peptide linker and connecting in-frame the first and second nucleic acid segments; wherein said β1 domain and said α1 domain form the peptide binding groove of the MHC class II heterodimer.

40. (new) The expression cassette of claim 39, further comprising:

a third nucleic acid segment encoding a third polypeptide segment comprising an antigenic peptide that associates with the peptide binding groove of the MHC class II heterodimer; and

a second linker segment encoding a second peptide linker and connecting in-frame the third and first nucleic acid segments.

41. (new) The expression cassette of claim 39, wherein the MHC class II β1 domain is from a human DR1β*1501 β1 domain.

42. (new) The expression cassette of claim 39, wherein the MHC class II α1 domain is from a human DRA*0101 α1 domain.

43. (new) The expression cassette of claim 39 or 40, wherein the first linker segment encodes a first peptide linker having the sequence GASAG (SEQ ID NO:29) or GGSGGS (SEQ ID NO:31).

44. (new) The expression cassette of claim 40, wherein the second linker segment encodes a second peptide linker having the sequence GASAG (SEQ ID NO:29) or GGSGGS (SEQ ID NO:31).

45. (new) The expression cassette of claim 40, wherein the third nucleic acid segment encodes an antigenic peptide capable of stimulating a CD4+ helper T cell-mediated immune response.

46. (new) The expression cassette of claim 39, further comprising an additional nucleic acid segment encoding a signal sequence.

47. (new) The expression cassette of claim 39, wherein the first linker segment encodes a first peptide linker of about 5 to about 25 amino acids.

48. (new) The expression cassette of claim 40, wherein the first linker segment and the second linker segment encode a first and a second peptide linker of about 5 to about 25 amino acids.

49. (new) The MHC class II heterodimer of claim 40, wherein the third nucleic acid segment encodes a peptide selected from the group consisting of SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:40, SEQ ID NO:39, and SEQ ID NO:33.

REMARKS

With this amendment, Applicants request entry of new claims 27-49 in the patent application. These claims replace originally filed claims 1-26.

The invention

The present invention relates to single chain, MHC class II heterodimers that are capable of associating with a peptide and presenting the peptide to a T cell receptor. In particular, the invention relates to the discovery that such MHC class II:peptide complexes can

be made in a soluble, single-chain form. These complexes can also comprise a preselected antigenic peptide, which is covalently linked via an amino acid linker to a single-chain MHC class II component. Alternatively, the single chain complex can be loaded with non-covalently linked peptide. In these complexes, the antigenic peptide binds to the antigen binding pocket of the MHC class II component and is specifically recognized by the target T-cell. These single-chain MHC II:peptide complexes can be used, e.g., to treat autoimmune diseases.

Status of the claims

Claims 27 and 28 recite "a soluble, fused MHC class II heterodimer comprising "a β1 and an α1 domain." Claims 39 and 40 recite an expression cassette encoding such a heterodimer. These amendments add no new matter. Support for these amendments can be found, e.g., in original claims 1-4 and 16-17 and in the specification on page 14, lines 5-21.

Claims 27 recites an MHC class II heterodimer that forms "a peptide binding groove that associates with an antigenic peptide." Claim 39 recites an expression cassette encoding such a heterodimer. This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 4, line 37 to page 5, line 1.

Claim 29 recites a human DR1β*1501 β1 domain and claim 30 recites a human DRA*0101 α1 domain. Claims 41 and 42 recite expression cassettes encoding such domains. This claim adds no new matter. Support for this amendment can be found, e g., in claim 5 as originally filed.

Claims 31 and 32 recite peptide linkers of about 5 to about 25 amino acids in length. Claims 47 and 48 recite an expression cassette encoding such linkers. These claims add no new matter. Support for these claims can be found, e.g., in the specification on page 9, lines 13-20.

Claims 33 and 34 recite a peptide linker having the sequence GASAG or GGGSGGS. Claims 43 and 44 recite expression cassettes encoding such linkers. These amendments add no new matter. Support for these amendments can be found, e.g., in the specification on page 5 lines 20-25.

Claim 35 recites an antigenic peptide capable of stimulating a CD4+ helper T cell-mediated immune response. Claim 45 recites an expression cassette encoding such a

peptide. This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 10, lines 2-20.

Claim 36 recites a third polypeptide segment that is a peptide selected from the group consisting of SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:40, SEQ ID NO:39, and SEQ ID NO:33. Claim 49 recites an expression cassette encoding such peptides. This claim adds no new matter. Support for this claim can be found, e.g., in claim 10 as originally filed.

Claims 37 and 38 recite "pharmaceutical compositions" comprising the MHC class II heterodimers of claims 1 and 2. These claims add no new matter. Support for these claims can be found in claim 20 as originally filed.

Claim 46 recites an expression cassette further encoding a signal sequence. This claim adds no new matter. Support for this claim can be found, e.g., in the specification on page 34, lines 5-12.

CONCLUSION

If the Examiner believes a telephone conference would aid in the prosecution of this case, please call the undersigned at 415-576-0200.

Respectfully submitted,

Annette S. Parent

Annette S. Parent
Reg. No. 42,058

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
ASP

APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The present application is a continuation of U.S. Serial No 09/261,811, filed March 3, 1999, and U.S. Serial No 08/657,581, filed June 7, 1996, and is a continuation-in-part of U.S. Serial No. 08/480,002, filed June 7, 1995, U.S. Serial No. 08/483,241, filed June 7, 1995 and U.S. Serial No. 08/482,133, filed June 7, 1995, and claims the benefit of U.S. Provisional Application No. 60/005,964, filed October 27, 1995, [which applications are pending] the disclosures of which are herein each incorporated by reference in their entirety.

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On MARCH 26, 2001

TOWNSEND and TOWNSEND and CREW LLP

By: Karen Dring

PATENT
Attorney Docket No.: 014058-005630US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: KINDSVOGEL *et al.*	Examiner: DeCloux, Amy
Application No.: 09/261,811	Art Unit: 1644
Filed: March 3, 1999	COMMUNICATION UNDER 37 C.F.R. §§ 1.821-1.825 AND AMENDMENT
For: IMMUNE MEDIATORS AND RELATED METHODS	

Box SEQUENCE
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Examiner's request to comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, 37 C.F.R. §§ 1.821-1.825, that accompanied the Office Action mailed December 4, 2000, Applicants submit herewith the required paper copy and computer readable copy of the Substitute Sequence Listing. Please amend the specification in adherence with 37 C.F.R. §§ 1.821-1.825 as follows.

In the Specification:

Please replace the paragraph beginning at page 5, line 20, with the following:

--Within still another embodiment the first linker DNA segment encodes GASAG (SEQ ID NO:29) or GGGGSGGGGSGGGGS (SEQ ID NO:36).--

Please replace the paragraph beginning at page 5, line 23, with the following:

--Within yet another embodiment the second linker DNA segment encodes GGSGG (SEQ ID NO:30) or GGGSGGS (SEQ ID NO:31).--

Please replace the paragraph beginning at page 5, line 29, with the following:

--Within another embodiment the peptide is selected from the group consisting of a mammalian GAD 65 peptide, (SEQ ID NO:47), (SEQ ID NO:89), (SEQ ID NO:40), (SEQ ID NO:39) and a mammalian myelin basic peptide(SEQ ID NO:33).--

Please replace the paragraph beginning at page 6, line 12, with the following:

--Within yet another embodiment the third linker DNA segment encodes GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:32).--

Please replace the paragraph beginning at page 35, line 1, with the following:

--Secretory signals include the α factor signal sequence (prepro sequence: Kurjan and Herskowitz, Cell 30:933-943, 1982; Kurjan et al., U.S. Patent No. 4,546,082; Brake, EP 116, 201), the *PHO5* signal sequence (Beck et al., WO 86/00637) , the *BAR1* secretory signal sequence (MacKay et al., U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the SUC2 signal sequence (Carlsen et al., Molecular and Cellular Biology 3: 439-447, 1983) , the a-l-antitrypsin signal sequence (Kurachi et al., Proc. Natl. Acad. Sci. USA 78: 6826-6830, 1981) , the a-2 plasmin inhibitor signal sequence (Tone et al., J. Biochem. (Tokyo) 102: 1033-1042, 1987) and the tissue plasminogen activator signal sequence (Pennica et al., Nature 301: 214-221, 1983). Alternately, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (European Journal of Biochemistry 133: 17-21, 1983; Journal of Molecular Biology 184: 99-105, 1985; Nucleic Acids Research 14: 4683-4690, 1986). Another signal sequence is the synthetic signal LaC212 spx (1-47) - ERLE (SEQ ID NO:42) described in WO 90/10075.--

Please replace the paragraph beginning at page 47, line 26, with the following:

--Plasmid pLJ13 contains the MHC Class II β chain (DRIβ *1501) signal sequence; a myelin basic protein encoding sequence (from bp 283 to 345, encoding amino acids DENPVVHFFKNIVTPRTPPPS 82 to 102) (SEQ. ID. NO. 33); a DNA sequence encoding a flexible linker represented by the amino acid sequence (GGGSGGS SEQ. ID. NO. 31); β1 region of Class II MHC DR1 β*1501 (SEQ ID NOS:120 and 121) (SEQ. ID. NO. 50) encoding sequence: a DNA sequence encoding a flexible linker, represented by the amino acid sequence (GASAG SEQ. ID. NO. 29); and an αl region of Class II MHC DRA*0101 (SEQ ID NOS:90 and 91) (SEQ. ID. NO. 51) encoding sequence. This plasmid was designed to direct secretion of a soluble, fused MHC heterodimer, denoted β1-α1, to which was attached, at the N terminus of β1, a myelin basic protein peptide that has been implicated in multiple sclerosis (Kamholz et al., Proc. Natl. Acad. Sci. USA 83:4962-66, 1986), thus forming a soluble, fused MHC heterodimer:peptide complex.--

Please replace the paragraph beginning at page 48, line 6, with the following:

--To construct pLJl3 (SEQ ID NOS:92 and 93) (SEQ. ID. NO. 49) , PCR was used to introduce a DNA sequence encoding MPB at the junction of the signal sequence and β1β2 sequence of the β chain of DRIβ*1501. This was followed by joining the MBP-containing β1 region to the αl region through a linker sequence which was introduced by PCR.--

Please replace the paragraph beginning at page 48, line 27, with the following:

--pZCEP was digested with Bam HI and XbaI, and a ~ 0.7 kb SacI-SSP I fragment, comprising the cDNA encoding a chain of DRA*0101, was isolated by agarose gel electrophoresis, and was inserted along with a polylinker sequence containing Bam HI-SacI and SSP I-XbaI ends. The resulting plasmid was designated pSL2.--

Please replace the paragraph beginning at page 48, line 33, with the following:

--A cloning site in the linker sequence was generated using PCR by amplifying a ~100 bp Hind III/Cla I fragment containing the signal sequence of Class II b DR1b*1501, to which a sequence encoding the first five amino acids (DPVVH; SEQ ID NO:43) of MBP (82-

104) was joined to the 3' end of the signal sequence. The DNA sequence encoding the amino acids VH was chosen to create a unique ApaLI site.--

Please replace the paragraph beginning at page 49, line 10, with the following:

--The fragments were digested with Hind Ill/Cla I and Cla I/Xba I, isolated by agarose gel electrophoresis, and inserted into Hind III/Xba I-digested pCZEP. The resulting shuttle plasmid was digested with ApaLI and BamHI, and oligonucleotides encoding the remaining portion of the MBP sequence (represented by the amino acid sequence FFKNIVTPRTPPPS; SEQ ID NO:44) and the start of the flexible linker GGGSG (SEQ ID NO:45) were inserted. The resulting construct contained the MBP sequence joined to the β1β2 sequence of DRlβ*1501 through an intervening linker. The resulting plasmid was designated pSL2l.--

Please replace the paragraph beginning at page 51, line 25, with the following:

--A 100 ml PCR reaction was prepared containing 1 ml signal sequence/MBP/linker/β1/linker fragment, 1 ml linker/al fragment, 200 pmol ZC7511 (SEQ. ID. NO. 1), 200 pmol ZC8196 (SEQ. ID. NO. 9), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94 °C for 1 minute, 50 °C for 1 minute, and 72 °C for 1 minute. The 5 amino acid 3' linker (GASAG SEQ. ID. NO. 29) of the signal sequence/MBP/linker/β1/linker fragment overlapped with the same 5 amino acid linker of the linker/α1 fragment joining the β1 and α1 domains in frame via the 5 amino acid linker. The resulting 730 bp MBP-β1α1 PCR product contained a 5' Hind III site followed by the DR1β*1501 β chain signal sequence, a 21 amino acid MHP peptide DENPVVHFFKNIVTPRTPPPS (SEQ. ID. NO. 33), an 8 amino acid flexible linker (GGGSGGSG; SEQ ID NO:46) attached to the N terminus of the DR1β*150l β1 domain which was attached to the N terminus of the DRA*0101, α1 domain by a 5 amino acid linker (GASAG SEQ. ID. NO. 29) and ending with a Xba I restriction site. The MBP β1α1 fragment was introduced into Hind III/XbaI pZCEP. A recombinant clone was identified by restriction and sequence analysis and given the designation pLJl3 (human MBP-β1α1).--

Please replace the paragraph beginning at page 54, line 6, with the following:

--1) The β1 domain (SEQ ID NOS:94 and 95) of the IA^{g7} NOD mouse β chain was isolated from the β2 domain and fused to linker fragments on both the 5' and 3' ends using PCR.--

Please replace the paragraph beginning at page 54, line 22, with the following:

--2) A GAD 65 peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47) and an additional linker fragment were added to the bl/linker fragment from 1 using PCR. In addition, a unique Bam HI site and a the last 16 nucleotides of the phi 10 coupler, adding a second ribosome binding site followed by a stop codon (RBS SEQ. ID. NO. 48) were also added to the 5' end of the GAD peptide to facilitate cloning and expression.--

Please replace the paragraph beginning at page 54, line 30, with the following:

--A 100 ml PCR reaction was prepared using 1 ml of eluted bl/linker fragment from above, 200 pmol ZC9473 (SEQ. ID. NO. 15), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9480 (SEQ. ID. NO. 18), 10 ml 10X polymerase buffer, 10 ml dNTPs, and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute. The fragments were designed so that all contained overlapping 5' and/or 3' segments, and could both anneal to their complement strand and serve as primers for the reaction. The final 15 3' nucleotides of ZC9499 (SEQ. ID. NO. 23) overlap with the first 15 nucleotides of the β1/linker fragment (ggaggctcaggagga) (SEQ. ID. NO. 35), seamlessly joining the GAD peptide in frame with the β1 domain through a 15 amino acid flexible linker (GGGGSGGGGSGGGGS) (SEQ ID. NO. 36) ZC9479 (SEQ. ID. NO. 17) served as the 5' primer, adding a Bam HI site followed by a RBS (SEQ. ID. NO. 48) to the 5' end of the GAD peptide sequence. A 15 nucleotide overlap (gaggatgattaaatg) (SEQ ID NO:49) between the 3' end of ZC9479 (SEQ. ID. NO. 17) and the first 15 nucleotides of ZC9473 (SEQ. ID. NO. 15) added the sites in frame with the peptide. The resulting 450 bp GAD/β1 fragment was isolated by low melt agarose gel electrophoresis.--

Please replace the paragraph beginning at page 55, line 16, with the following:

--3) The αl domain (SEQ ID NOS:96 and 97) of the IA^{g7} was isolated from the 0.2 domain, and fused to a linker fragment on the 5' end and a serine residue, followed by a Spe I and Eco RI site, on the 3' end using PCR.--

Please replace the paragraph beginning at page 55, line 32, with the following:

--4) To complete the construct, a final 100 ml PCR reaction was prepared containing 2 ml GAD/β1 fragment from 2), 2 ml α1/linker fragment from 3), 200 pmol ZC9479 (SEQ. ID. NO. 17) , 200 pmol ZC9493 (SEQ. ID. NO. 20) , 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The 5 amino acid 3' linker (GGSGG SEQ. ID. NO. 30) of the GAD/βl fragment overlapped with the 5 amino acid linker of the α1/linker fragment joining the β1 and αl domains in frame via the 5 amino acid linker. The resulting GAD-β1α1 PCR product contained a 5' Bam HI site followed by a RBS (SEQ. ID. NO. 48), a 20 amino acid GAD65 peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS (SEQ. ID. NO. 36) attached to the N terminus of the β1 domain of IA^{g7}, which was attached to the N terminus of the α1 domain of IA^{g7} by a 5 amino acid linker (GGSGG SEQ. IS. NO. 30) and ending with a Spe I and Eco RI restriction site. The GAD-β1α1 fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragments were then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A correct recombinant clone was identified by restriction and sequence analysis and given the designation pLJl8 (GAD-β1α1 IA^{g7}) (SEQ ID NOS:98 and 99).--

Please replace the paragraph beginning at page 56, line 23, with the following:

--The β1 domain (SEQ ID NOS:100 and 101) of IA^{S} was isolated from the β2 domain and fused to linker fragments on both the 5' and 3' ends using PCR.--

Please replace the paragraph beginning at page 57, line 31, with the following:

--3) The αl domain (SEQ ID NOS:102 and 103) of IA^S was isolated from the α2 domain and fused to a linker fragment on the 5' end, and a serine residue, followed by a Spe I and Eco RI site on the 3' end, using PCR.--

Please replace the paragraph beginning at page 58, line 10, with the following:

--4) To complete the construct, a final 100 ml PCR reaction was prepared containing 2 ml MBP/IA^S β1 fragment from 2), 2 ml IA^S αl/linker fragment from 3), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9496 (SEQ. ID. NO. 21) 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The 5 amino acid 3' linker (GGSGG SEQ. ID. NO. 30) of the MBP/IA^S β1 fragment, overlapped with the same 5 amino acid linker of the IA^S β1/linker fragment, joining the IA^S α1 and IA^S αl domains in frame, via the 5 amino acid linker. The resulting 673 bp MBP-β1α1 IA^S PCR product contained a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP SEQ. ID. NO. 37), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) attached to the N terminus of the IA^S β1 domain, which was attached to the N terminus of the IA^S αl domain by a 5 amino acid linker (GGSGG SEQ ID NO 30), and ending with a Spe I and Eco RI restriction site. The MBP β1α1 fragment was restriction digested with Bam HI and Eco RI, and isolated by low melt agarose gel electrophoresis. The restriction digested fragments were then subcloned onto a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ19 (MBP β1α1 IA^S) (SEQ ID NOS:104 and 105).--

Please replace the paragraph beginning at page 59, line 32, with the following:

--A 100 ml PCR reaction was prepared containing 100 ng full length lineralized I-A^{g7} β chain (pLJ12), 200 pmol ZC9721 (SEQ. ID. NO. 26), 200 pmol ZC9521 (SEQ. ID. NO. 24), 5 ml l0X polymerase buffer, 5 ml dNTPs and 2.5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. An I-A^{g7} linker/β2 fragment, comprising the β2 domain (SEQ ID NOS:106 and 107), with a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) fused to the 5' end, and stop

codon and Eco RI restriction site fused to the 3' end, was obtained. A band of the predicted size was isolated by low melt agarose gel electrophoresis.--

Please replace the paragraph beginning at page 60, line 6, with the following:

--3) The α1α2 domain (SEQ ID NOS:108 and 109) of the I-A^{g7} was fused to β2 domain of I-A^{g7} using PCR. The 15 amino acid linker sequence on the 3' end of the α1α2 fragment overlapped completely with the same 15 amino acid sequence on the 5' end of the β2 fragment, joining the domains in frame, via a flexible linker.--

Please replace the paragraph beginning at page 60, line 26, with the following:

--4) To complete the construct a final 100 ml PCR reaction was prepared containing 5 ml GAD-β1αl fragment from A-4 above, 5 ml I-A^{g7} linker/α1α2/linker/β2 fragment from 3), 200 pmol ZC9521 (SEQ. ID. NO. 24), 200 pmol ZC9479 (SEQ. ID. NO. 17), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 30 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes. The entire linker/al portions of both the GAD-β1α1 and linker/α1α2/linker/β2 fragments overlapped, joining the I-A^{g7} β1 and I-A^{g7} α1α2/linker/β2 domains in frame, via the 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30). The resulting GAD-β1α1α2β2 I-A^{g7} PCR product contained a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 20 amino acid GAD peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36), attached to the N terminus of the I-A^{g7} β1 domain, which was attached to the N terminus of the α1α2 domain by a 5 amino acid flexible linker (GGSGG, SEQ. ID. NO. 30), and ending with the β2 domain, and an Eco RI restriction site. The GAD-β1 α1α2β2 fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ23 (GAD-β1α1α2β2 I-A^{g7}) (SEQ ID NOS:110 and 111).--

Please replace the paragraph beginning at page 62, line 1, with the following:

--A 100 ml PCR reaction was prepared containing 100 ng full length lineralized IA^S β chain (p40553) , 200 pmol ZC9721 (SEQ. ID. NO. 28), 200 pmol ZC9521 (SEQ. ID. NO. 24) , 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. An IA^S linker/β2 fragment, comprising the 105 amino acid β2 domain (SEQ ID NOS:112 and 113), with a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) fused to the 5' end, and stop codon, and Eco RI restriction site, fused to the 3' end, was obtained. A band of the predicted size, 374 bp, was isolated by low melt agarose gel electrophoresis.--

Please replace the paragraph beginning at page 62, line 35, with the following:

--4) To complete the construct a final 100 ml PCR reaction was prepared containing 2 ml MBP-β1α1 fragment from B-4 above, 2 ml IA^S linker/α1α2/linker/β2 fragment from 3) , 200 pmol ZC9521 (SEQ. ID. NO. 24), 200 pmol ZC9479 (SEQ. ID. NO. 17), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 30 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. The entire linker/α1 portions of both the MBP-β1α1 and linker/ α1α2/linker/β2 fragments overlapped, joining the IA^S β1 and IA^S α1α2/linker/β2 domains, in frame via the 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30). The resulting 1360 bp MBP-β1α1α2β2 IA^S PCR product contained, a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP SEQ. ID. NO. 37), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36), attached to the N terminus of the IA^S β1 domain, which was attached to the N terminus of the full length IA^S α domain by a 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30), and ending with the β2 domain and an Eco RI restriction site. The MBP β1α1α2β2 fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ20 (MBP $\beta1\alpha1\alpha2\beta_2$ IA^S) (SEQ ID NOS:114 and 115).--

Please replace the paragraph beginning at page 63, line 32, with the following:

--1) The α1α2 domain of the I-A^S (SEQ ID NOS:116 and 117) was fused to a 25 amino acid linker on the 5' end, and a stop codon and Spe I and Eco RI restriction sites on the 3', end using PCR.--

Please replace the paragraph beginning at page 64, line 25, with the following:

--There was a 12 amino acid overlap (GGGGSGGGGSGG SEQ. ID. NO. 38) between the 5' end of the 25 amino acid linker, of the linker/α1α2 fragment, and the 3' end of ZC9499 (SEQ. ID. NO. 23). ZC9499 (SEQ. ID. NO. 23 added a Bam HI restriction site, RBS (SEQ. ID. NO. 48), and MBP peptide(FFKNIVTPRTPPP (SEQ. ID. NO. 37), to the 5' end of the 25 amino acid flexible linker. ZC9479 (SEQ. ID. NO. 17) served as a 5' primer, overlapping the first 32 nucleotides of ZC9499 (SEQ. ID. NO. 23). The resulting 743 bp MBP- α1α2 IA^S PCR product contained, a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP (SEQ. ID. NO. 37), a 25 amino acid flexible linker (GGGGSGGGGSGGGGSGGGGSGGGGS SEQ. ID. NO. 32) attached to the N terminus of the IA^S α1α2 domain, which ended with a Spe I and Eco RI restriction site. The MHP- α1α2 fragment was restriction digested with Bam HI and Eco RI, and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ21 (MBP-α1α2 IA^S) (SEQ ID NOS:118 and 119).--

Please replace the paragraph beginning at page 71, line 34, with the following:

--Four individual T cell lines derived from one HLA-DRBl*0404 patient (ThHo) were used to map the 74 synthetic GAD peptides, overlapping sets of 20 mers, that span the entire length of GAD 65 (SEQ ID NO:47). Antigen presenting cells, BLS-DRBl*0404 and/or BLS-DRB1*0401 (Kovats et al, *J. Exp. Med.* 179:2017-22, 1994), were loaded with peptide by incubating with peptide (about 50 mg/ml) for at least 5 hours. Reactivity of T-cells was determined as above. One peptide, hGAD 33 (PGGAISNMYAMMIARFKNFP SEQ. ID. NO. 40) stimulated 3 or the 4 lines with BLS-Bl*0404. COOH terminal truncations of this peptide from 20 amino acids to an 11 amino acid fragment (PGGAISNMYAM SEQ. ID. NO. 39) when

presented by either BLS-B1*0404 or BLS-DRBI*040l, stimulated only one or the T-cell lines. A 10 amino acid fragment (PGGAISNMYA SEQ. ID. NO. 41) stimulated the same T-cell line only when presented by BLS-Bl*0404. This methodology quickly identifies peptide and HLA restriction of T-cell lines and clones as well as identifying GAD epitopes which stimulate T-cell lines derived from a prediabetic donor.--

Please replace the paragraph beginning at page 72, line 20, with the following:

--Peptides amidated at the C terminus were synthesized by solid phase peptide synthesis (SPPS) using Fmoc chemistry. Chemicals used in the synthesis were obtained from Nova Biochem (La Jolla, CA) . The peptide was assembled on Rink amide MBHA resin (0.25 millimolar scale) starting from the C terminal end by using a 432A Applied Biosystems, Inc. (Foster City, CA) automated peptide synthesizer and solid phase strategy. The synthesis required double coupling to ensure completion of the coupling reaction, and HBtu-HOBt coupling chemistry was used. Bolded residues required at least double coupling (SRLSKVAPVIKARMMEYGTT-NH2) (SEQ ID NO:50). Each cycle included Fmoc deprotection of amine from the amino acid residue on the resin, and coupling of incoming Fmoc-amino acid. After successful assembly of the peptide, the resin was washed with dichloromethane and dried under vacuum for two hours. The peptide resin was resuspended in 10 ml trifluoroacetic acid (TFA) containing 1 ml of 4-methoxybenzenethiol and 0.7 g of 4-methylmercaptophenol as scavengers. This suspension was gently mixed at room temperature for 2 hours, then filtered through a PTFE filter, and the filtrate was collected in a capped glass bottle containing 1 liter organic solvent mixture (pentane:acetone = 4:1). The white precipitate was allowed to settle at room temperature for 1-2 hours, after which the crude precipitated peptide was isolated by centrifugation. The crude peptide was washed three times with the organic solvent mixture and dried under vacuum overnight.--

Please replace the paragraph beginning at page 76, line 12, with the following:

--A series of C-terminal amidated GAD 65 (SEQ ID NO:50) peptides were synthesized where one or more N-terminal or C-terminal amino acids were systematically truncated (Table 3).--

Please replace the paragraph (Table 3) beginning at page 76, line 17, with the following:

--Table 3 Truncated GAD65 peptides from amino acid 524 (1) to amino acid 543 (20). All peptides are amidated at the C-terminus.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SEQ ID NO:
S	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	50
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	51
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	52
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	53
				K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	54
					V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	55
						A	P	V	I	K	A	R	M	M	E	Y	G	T	T	56
							P	V	I	K	A	R	M	M	E	Y	G	T	T	57
								V	I	K	A	R	M	M	E	Y	G	T	T	58
									I	K	A	R	M	M	E	Y	G	T	T	59
										K	A	R	M	M	E	Y	G	T	T	60
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T		61
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G			62
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y				63
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E					64
	S	L	S	K	V	A	P	V	I	K	A	R	M	M						65
	S	L	S	K	V	A	P	V	I	K	A	R	M							66
	S	L	S	K	V	A	P	V	I	K	A	R								67
	S	L	S	K	V	A	P	V	I	K	A									68
	S	L	S	K	V	A	P	V	I	K										69
	S	L	S	K	V	A	P	V	I											70

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Please replace the paragraph (Table 4) beginning at page 78, line 1, with the following:

--Table 4. Truncated GAD65 core pectides. The C-terminus of each peptide is amidated. 1 is amino acid 524, 20 is amino acid 543.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SEQ ID NO:
			S	K	V	A	P	V	I	K	A	R	M	M	E						71
				K	V	A	P	V	I	K	A	R	M	M	E						72
					V	A	P	V	I	K	A	R	M	M	E						73
						A	P	V	I	K	A	R	M	M	E						74
							P	V	I	K	A	R	M	M	E						75
			S	K	V	A	P	V	I	K	A	R	M	M							76
			S	K	V	A	P	V	I	K	A	R	M								77
			S	K	V	A	P	V	I	K	A	R									78
			S	K	V	A	P	V	I	K	A										79
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				80
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y					81
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E						82
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				83
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y					84
		L	S	K	V	A	P	V	I	K	A	R	M	M	E						85
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				86
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y					87
			S	K	V	A	P	V	I	K											88

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Please replace the paragraph beginning at page 85, line 40, with the following:

--One hundred microliters of the cell-protease inhibitor mixture was added to each well of a 96-well round-bottom plate (Costar, Pleasanton, CA). Fixed NOD cells were co-incubated with biotinylated, C-terminal amidated GAD65 peptide at a concentration of 10,000 nM and unlabeled, Ala scan peptides at concentrations of 100,000, 1,000 and 10 nM for 12-20 hours at 37°C. Mouse serum albumin (MSA), a known allele-specific peptide (SEQ ID NO:89) with high affinity for I-A^{g7}, was used as a positive control, and Eα, which binds to I-A^{d} but not to I-A^{g7}, served as a negative control (Reich et al., J. Immunol. 154: 2279-88, 1994). Following incubation, the plates were vortexed and centrifuged in a Beckman GA-6R centrifuge for 10 minutes at 1500 rpm (Beckman, Fullerton, CA). The supernatant was removed, and the cells were lysed in 60 µl/well of NP-40 lysis buffer (0.5% NP4O, 0.15 M NaCl, 50 mM Tris, pH 8.0, 0.01% sodium azide, and 1:50 dilutions of the protease inhibitor stocks D, E and F (Table 3). The cells were incubated on ice for 30 minutes, with mixing every 15 minutes, followed by centrifuging for 10 minutes at 1500 rpm to obtain a clear lysate.--

Please replace the paragraph beginning at page 91, line 6, with the following:

--Newly diabetic NOD mice were irradiated (730 rad) and randomly divided into 4 treatment groups, and splenocytes were isolated as described above. Non-diabetic 7-8 week old, NOD recipient mice were divided into 4 groups. Group one received 1 x 10^7 splenocytes, injected intravenously. Six hours following the injection the mice received a second intravenous injection of either saline, 10 µg/mouse C-terminal amidated GAD65 peptide, or 10, 5, or 1 µg/mouse C-terminal amidated GAD65 peptide-MHC complex. Group two received 2 x 10^7 splenocytes, followed by injections with either saline, 10 µg/mouse C-terminal amidated GAD65 peptide-MHC complex, or 5 µg/mouse MSA-MHC complex. Group three received 1 x 10^7 splenocytes and injections of either saline, 10 µg/mouse C-terminal amidated GAD65 or 200 µg/mouse 10.2.16, an anti-class II antibody. Group four received 1 x 10^7 splenocytes followed by injection with either saline, 20 µg/mouse C-terminal amidated GAD65 peptide, or 1, 5 or 10 µg/mouse C-terminal amidated GAD65 peptide-MHC complex. Group four mice received only two treatments with peptide or peptide-MHC complex, one on day 0 and a second on day 4. All other groups received further treatments on days 8 and 12. The mice were tested for the onset of diabetes by urine analysis. On the day the first animal showed overt signs of diabetes, as

determined by urine and blood glucose levels, mice from each of the treatment groups were randomly selected, and urine and blood glucose levels determined for all selected mice, which were then sacrificed, and spleens and pancreases removed for immunohistochemical analysis. Saline-treated mice developed diabetes within about 12-20 days. Group one mice, which received four treatments of 10 μg peptide-MHC complex, had no significant development of disease by day 30, and did not develop disease until day 75. Those receiving 5 μg peptide-MHC complex had stabilized at 40% diseased mice by day 30, with a gradual increase in disease onset up to day 80, when there was 100% disease among the mice. Those mice in group four, which received only two treatments of peptide-MHC complex, experienced some delayed onset of disease, i.e., less than 50% of those mice receiving 10 μg of peptide-MHC had developed disease by day 30. Blocking with anti-MHC antibody in group three delayed the onset of disease, but provided less protection, i.e., over 75% of those mice receiving 10 μg peptide alone had developed disease by day 30. The C-terminal amidated GAD65 (SEQ ID NO:50) peptide alone accelerated the onset of diabetes in this adoptive transfer model, while the peptide-MHC complex prevented onset of disease.--

Please cancel the present "SEQUENCE LISTING", pages 93-135, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 61, at the end of the application.

REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-121, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Annette S. Parent
Reg. No. 42,058

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
ASP:dmw

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 20 of page 5 has been amended as follows:

Within still another embodiment the first linker DNA segment encodes ~~is~~ GASAG (SEQ ID NO:29) ~~(SEQ. ID. NO. 29)~~ or GGGGSGGGGSGGGGS (SEQ ID NO:36) ~~(SEQ. ID. NO. 36)~~.

Paragraph beginning at line 23 of page 5 has been amended as follows:

Within yet another embodiment the second linker DNA segment encodes ~~is~~ GGSGG (SEQ ID NO:30) ~~(SEQ. ID. NO. 30)~~ or GGGSGGS (SEQ ID NO:31) ~~(SEQ. ID. NO. 31)~~.

Paragraph beginning at line 29 of page 5 has been amended as follows:

Within another embodiment the peptide is selected from the group consisting of a mammalian GAD 65 peptide, (SEQ ID NO:47) ~~(SEQ ID NO: 59)~~, (SEQ ID NO:89) ~~(SEQ. ID. NO. 61)~~, (SEQ ID NO:40) ~~(SEQ ID NO: 40)~~, (SEQ ID NO:39) ~~(SEQ. ID. NO. 39)~~ and a mammalian myelin basic peptide (SEQ ID NO:33) ~~(SEQ. ID. NO. 33)~~.

Paragraph beginning at line 12 of page 6 has been amended as follows:

Within yet another embodiment the third linker DNA segment encodes ~~is~~ GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:32) ~~(SEQ. ID. NO. 32)~~.

Paragraph beginning at line 1 of page 35 has been amended as follows:

Secretory signals include the α factor signal sequence (prepro sequence: Kurjan and Herskowitz, Cell 30:933-943, 1982; Kurjan et al., U.S. Patent No. 4,546,082; Brake, EP 116, 201), the *PHO5* signal sequence (Beck et al., WO 86/00637) , the *BAR1* secretory signal sequence (MacKay et al., U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the SUC2

signal sequence (Carlsen et al., Molecular and Cellular Biology 3: 439-447, 1983) , the a-l-antitrypsin signal sequence (Kurachi et al., Proc. Natl. Acad. Sci. USA 78: 6826-6830, 1981) , the a-2 plasmin inhibitor signal sequence (Tone et al., J. Biochem. (Tokyo) 102: 1033-1042, 1987) and the tissue plasminogen activator signal sequence (Pennica et al., Nature 301: 214-221, 1983). Alternately, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (European Journal of Biochemistry 133: 17-21, 1983; Journal of Molecular Biology 184: 99-105, 1985; Nucleic Acids Research 14: 4683-4690, 1986). Another signal sequence is the synthetic signal LaC212 spx (1-47) - ERLE (SEQ ID NO:42) described in WO 90/10075.

Paragraph beginning at line 26 of page 47 has been amended as follows:

Plasmid pLJ13 contains the MHC Class II β chain (DRIβ *1501) signal sequence; a myelin basic protein encoding sequence (from bp 283 to 345, encoding amino acids DENPVVHFFKNIVTPRTPPPS 82 to 102) (SEQ. ID. NO. 33); a DNA sequence encoding a flexible linker represented by the amino acid sequence (GGGSGGS SEQ. ID. NO. 31); β1 region of Class II MHC DR1 β*1501 (SEQ ID NOS:120 and 121) ~~(SEQ. ID. NO. 50)~~ encoding sequence: a DNA sequence encoding a flexible linker, represented by the amino acid sequence (GASAG SEQ. ID. NO. 29); and an αI region of Class II MHC DRA*0101 (SEQ ID NOS:90 and 91) ~~(SEQ. ID. NO. 51)~~ encoding sequence. This plasmid was designed to direct secretion of a soluble, fused MHC heterodimer, denoted β1-α1, to which was attached, at the N terminus of β1, a myelin basic protein peptide that has been implicated in multiple sclerosis (Kamholz et al., Proc. Natl. Acad. Sci. USA 83:4962-66, 1986), thus forming a soluble, fused MHC heterodimer:peptide complex.

Paragraph beginning at line 6 of page 48 has been amended as follows:

To construct pLJl3 (SEQ ID NOS:92 and 93) ~~(SEQ. ID. NO. 49)~~ , PCR was used to introduce a DNA sequence encoding MPB at the junction of the signal sequence and β1β2 sequence of the β chain of DRIβ*1501. This was followed by joining the MBP-containing β1 region to the αI region through a linker sequence which was introduced by PCR.

Paragraph beginning at line 27 of page 48 has been amended as follows:

pZCEP was digested with Bam HI and XbaI, and a ~ 0.7 kb SacI-SSP I fragment, comprising the cDNA encoding a chain of DRA*0101, was isolated by agarose gel electrophoresis, and was inserted along with a polylinker sequence containing Bam HI-SacI and SSP I-XbaI ends ~~(SEQ. ID. NO.)~~. The resulting plasmid was designated pSL2.

Paragraph beginning at line 33 of page 48 has been amended as follows:

A cloning site in the linker sequence was generated using PCR by amplifying a ~100 bp Hind III/Cla I fragment containing the signal sequence of Class II b DR1b*1501, to which a sequence encoding the first five amino acids (DPVVH; SEQ ID NO:43) of MBP (82-104) was joined to the 3' end of the signal sequence. The DNA sequence encoding the amino acids VH was chosen to create a unique ApaLI site.

Paragraph beginning at line 10 of page 49 has been amended as follows:

The fragments were digested with Hind III/Cla I and Cla I/Xba I, isolated by agarose gel electrophoresis, and inserted into Hind III/Xba I-digested pCZEP. The resulting shuttle plasmid was digested with ApaLI and BamHI, and oligonucleotides encoding the remaining portion of the MBP sequence (represented by the amino acid sequence FFKNIVTPRTPPPS; SEQ ID NO:44) and the start of the flexible linker GGGSG (SEQ ID NO:45) were inserted. The resulting construct contained the MBP sequence joined to the β1β2 sequence of DRIβ*1501 through an intervening linker. The resulting plasmid was designated pSL2I.

Paragraph beginning at line 25 of page 51 has been amended as follows:

A 100 ml PCR reaction was prepared containing 1 ml signal sequence/MBP/linker/β1/linker fragment, 1 ml linker/al fragment, 200 pmol ZC7511 (SEQ. ID. NO. 1), 200 pmol ZC8196 (SEQ. ID. NO. 9), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94 °C for 1 minute, 50 °C for 1 minute, and 72 °C for 1 minute. The 5 amino acid 3' linker (GASAG SEQ. ID. NO. 29) of the signal sequence/MBP/linker/β1/linker fragment overlapped with the same 5 amino acid linker of

the linker/α1 fragment joining the β1 and α1 domains in frame via the 5 amino acid linker. The resulting 730 bp MBP-β1α1 PCR product contained a 5' Hind III site followed by the DR1β*1501 β chain signal sequence, a 21 amino acid MHP peptide DENPVVHFFKNIVTPRTPPPS (SEQ. ID. NO. 33), an 8 amino acid flexible linker (GGGSGGSG; SEQ ID NO:46) attached to the N terminus of the DR1β*1501 β1 domain which was attached to the N terminus of the DRA*0101, α1 domain by a 5 amino acid linker (GASAG SEQ. ID. NO. 29) and ending with a Xba I restriction site. The MBP β1α1 fragment was introduced into Hind III/XbaI pZCEP. A recombinant clone was identified by restriction and sequence analysis and given the designation pLJI3 (human MBP-β1α1).

Paragraph beginning at line 6 of page 54 has been amended as follows:

1) The β1 domain (SEQ ID NOS:94 and 95) ~~(SEQ. ID. NO. 43)~~ of the IA^{g7} NOD mouse β chain was isolated from the β2 domain and fused to linker fragments on both the 5' and 3' ends using PCR.

Paragraph beginning at line 22 of page 54 has been amended as follows:

2) A GAD 65 peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47) ~~(SEQ. ID. NO. 59)~~ and an additional linker fragment were added to the bl/linker fragment from 1 using PCR. In addition, a unique Bam HI site and a the last 16 nucleotides of the phi 10 coupler, adding a second ribosome binding site followed by a stop codon (RBS SEQ. ID. NO. 48) were also added to the 5' end of the GAD peptide to facilitate cloning and expression.

Paragraph beginning at line 30 of page 54 has been amended as follows:

A 100 ml PCR reaction was prepared using 1 ml of eluted bl/linker fragment from above, 200 pmol ZC9473 (SEQ. ID. NO. 15), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9480 (SEQ. ID. NO. 18), 10 ml 10X polymerase buffer, 10 ml dNTPs, and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute. The fragments were designed so that all contained overlapping 5' and/or 3' segments, and could both anneal to their complement strand and serve as primers for the reaction. The final 15 3' nucleotides of ZC9499 (SEQ. ID. NO. 23) overlap with the first 15

nucleotides of the β1/linker fragment (ggaggctcaggagga) (SEQ. ID. NO. 35), seamlessly joining the GAD peptide in frame with the β1 domain through a 15 amino acid flexible linker (GGGGSGGGGSGGGGS) (SEQ ID. NO. 36) ZC9479 (SEQ. ID. NO. 17) served as the 5' primer, adding a Bam HI site followed by a RBS (SEQ. ID. NO. 48) to the 5' end of the GAD peptide sequence. A 15 nucleotide overlap (gaggatgattaaatg) (SEQ ID NO:49) between the 3' end of ZC9479 (SEQ. ID. NO. 17) and the first 15 nucleotides of ZC9473 (SEQ. ID. NO. 15) added the sites in frame with the peptide. The resulting 450 bp GAD/β1 fragment was isolated by low melt agarose gel electrophoresis.

Paragraph beginning at line 16 of page 55 has been amended as follows:

3) The αl domain (SEQ ID NOS:96 and 97) ~~(SEQ. ID. NO. 44)~~ of the IA^{g7} was isolated from the 0.2 domain, and fused to a linker fragment on the 5' end and a serine residue, followed by a Spe I and Eco RI site, on the 3' end using PCR.

Paragraph beginning at line 32 of page 55 has been amended as follows:

4) To complete the construct, a final 100 ml PCR reaction was prepared containing 2 ml GAD/β1 fragment from 2), 2 ml α1/linker fragment from 3), 200 pmol ZC9479 (SEQ. ID. NO. 17) , 200 pmol ZC9493 (SEQ. ID. NO. 20) , 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The 5 amino acid 3' linker (GGSGG SEQ. ID. NO. 30) of the GAD/βl fragment overlapped with the 5 amino acid linker of the α1/linker fragment joining the β1 and αl domains in frame via the 5 amino acid linker. The resulting GAD-β1α1 PCR product contained a 5' Bam HI site followed by a RBS (SEQ. ID. NO. 48), a 20 amino acid GAD65 peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47) ~~(SEQ. ID. NO.)~~, a 15 amino acid flexible linker (GGGGSGGGGSGGGGS (SEQ. ID. NO. 36) attached to the N terminus of the β1 domain of IA^{g7}, which was attached to the N terminus of the α1 domain of IA^{g7} by a 5 amino acid linker (GGSGG SEQ. IS. NO. 30) and ending with a Spe I and Eco RI restriction site. The GAD-β1α1 fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragments were then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A correct recombinant clone was identified by restriction and sequence analysis and given the designation pLJl8 (GAD-β1α1 lA^{g7}) (SEQ ID NOS:98 and 99) ~~SEQ. ID. NO. 42~~).

Paragraph beginning at line 23 of page 56 has been amended as follows:

The β1 domain (SEQ ID NOS:100 and 101) ~~(SEQ. ID. NO. 46)~~ of IA^S was isolated from the β2 domain and fused to linker fragments on both the 5' and 3' ends using PCR.

Paragraph beginning at line 31 of page 57 has been amended as follows:

3) The αl domain (SEQ ID NOS:102 and 103) ~~(SEQ. ID. NO. 47)~~ of IA^S was isolated from the α2 domain and fused to a linker fragment on the 5' end, and a serine residue, followed by a Spe I and Eco RI site on the 3' end, using PCR.

Paragraph beginning at line 10 of page 58 has been amended as follows:

4) To complete the construct, a final 100 ml PCR reaction was prepared containing 2 ml MBP/IA^S β1 fragment from 2), 2 ml IA^S αl/linker fragment from 3), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9496 (SEQ. ID. NO. 21) 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The 5 amino acid 3' linker (GGSGG SEQ. ID. NO. 30) of the MBP/IA^S β1 fragment, overlapped with the same 5 amino acid linker of the IA^S β1/linker fragment, joining the IA^S α1 and IA^S αl domains in frame, via the 5 amino acid linker. The resulting 673 bp MBP-β1α1 IA^S PCR product contained a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP SEQ. ID. NO. 37), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) attached to the N terminus of the IA^S β1 domain, which was attached to the N terminus of the IA^S αl domain by a 5 amino acid linker (GGSGG SEQ ID NO 30), and ending with a Spe I and Eco RI restriction site. The MBP β1α1 fragment was restriction digested with Bam HI and Eco RI, and isolated by low melt agarose gel electrophoresis. The restriction digested fragments were then subcloned onto a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJl9 (MBP β1α1 IA^S) (SEQ ID NOS:104 and 105) ~~SEQ. ID. NO. 45~~).

Paragraph beginning at line 32 of page 59 has been amended as follows:

A 100 ml PCR reaction was prepared containing 100 ng full length lineralized I-A^{g7} β chain (pLJ12), 200 pmol ZC9721 (SEQ. ID. NO. 26), 200 pmol ZC9521 (SEQ. ID. NO. 24), 5 ml l0X polymerase buffer, 5 ml dNTPs and 2.5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. An I-A^{g7} linker/β2 fragment, comprising the β2 domain (SEQ ID NOS:106 and 107) ~~(SEQ. ID. NO. 58)~~, with a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) fused to the 5' end, and stop codon and Eco RI restriction site fused to the 3' end, was obtained. A band of the predicted size was isolated by low melt agarose gel electrophoresis.

Paragraph beginning at line 6 of page 60 has been amended as follows:

3) The α1α2 domain (SEQ ID NOS:108 and 109) ~~(SEQ. ID. NO. 57)~~of the I-A^{g7} was fused to β2 domain of I-A^{g7} using PCR. The 15 amino acid linker sequence on the 3' end of the α1α2 fragment overlapped completely with the same 15 amino acid sequence on the 5' end of the β2 fragment, joining the domains in frame, via a flexible linker.

Paragraph beginning at line 26 of page 60 has been amended as follows:

4) To complete the construct a final 100 ml PCR reaction was prepared containing 5 ml GAD-β1αl fragment from A-4 above, 5 ml I-A^{g7} linker/α1α2/linker/β2 fragment from 3), 200 pmol ZC9521 (SEQ. ID. NO. 24), 200 pmol ZC9479 (SEQ. ID. NO. 17), 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 30 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes. The entire linker/al portions of both the GAD-β1α1 and linker/α1α2/linker/β2 fragments overlapped, joining the I-A^{g7} β1 and I-A^{g7} α1α2/linker/β2 domains in frame, via the 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30). The resulting GAD-β1α1α2β2 I-A^{g7} PCR product contained a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 20 amino acid GAD peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47) ~~(SEQ. ID. NO. 59)~~, a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36), attached to the N terminus of the I-A^{g7} β1 domain, which was attached to the N terminus of the α1α2 domain by a 5 amino acid flexible linker (GGSGG, SEQ. ID. NO. 30), and ending with the β2 domain, and an Eco RI restriction site. The GAD-β1 α1α2β2 fragment was restriction digested with Bam HI and Eco RI and

isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ23 (GAD-β1α1α2β2 I-A^{87}) (SEQ ID NOS:110 and 111) ~~SEQ. ID. NO. 56~~).

Paragraph beginning at line 1 of page 62 has been amended as follows:

A 100 ml PCR reaction was prepared containing 100 ng full length lineralized IA^S β chain (p40553) , 200 pmol ZC9721 (SEQ. ID. NO. 28), 200 pmol ZC9521 (SEQ. ID. NO. 24) , 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. An IA^S linker/β2 fragment, comprising the 105 amino acid β2 domain (SEQ ID NOS:112 and 113) ~~(SEQ. ID. NO. 55)~~, with a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) fused to the 5' end, and stop codon, and Eco RI restriction site, fused to the 3' end, was obtained. A band of the predicted size, 374 bp, was isolated by low melt agarose gel electrophoresis.

Paragraph beginning at line 35 of page 62 has been amended as follows:

4) To complete the construct a final 100 ml PCR reaction was prepared containing 2 ml MBP-β1α1 fragment from B-4 above, 2 ml IA^S linker/α1α2/linker/β2 fragment from 3) , 200 pmol ZC9521 (SEQ. ID. NO. 24), 200 pmol ZC9479 (SEQ. ID. NO. 17), 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 30 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. The entire linker/αl portions of both the MBP-β1α1 and linker/ α1α2/linker/β2 fragments overlapped, joining the IA^S β1 and IA^S α1α2/linker/β2 domains, in frame via the 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30). The resulting 1360 bp MBP-β1α1α2β2 IA^S PCR product contained, a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP SEQ. ID. NO. 37), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36), attached to the N terminus of the IA^S β1 domain, which was attached to the N terminus of the full length IA^S α domain by a 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30), and ending with the β2 domain and an Eco RI restriction site. The MBP β1α1α2β2 fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression

vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ20 (MBP $\beta 1\alpha 1\alpha 2\beta_2$ IA^S) (SEQ ID NOS:114 and 115) ~~SEQ. ID. NO. 54)~~.

Paragraph beginning at line 32 of page 63 has been amended as follows:

1) The $\alpha 1\alpha 2$ domain of the I-A^S (SEQ ID NOS:116 and 117) ~~(SEQ. ID. NO. 53)~~ was fused to a 25 amino acid linker on the 5' end, and a stop codon and Spe I and Eco RI restriction sites on the 3', end using PCR.

Paragraph beginning at line 25 of page 64 has been amended as follows:

There was a 12 amino acid overlap (GGGGSGGGGSGG SEQ. ID. NO. 38) between the 5' end of the 25 amino acid linker, of the linker/$\alpha 1\alpha 2$ fragment, and the 3' end of ZC9499 (SEQ. ID. NO. 23). ZC9499 (SEQ. ID. NO. 23 added a Bam HI restriction site, RBS (SEQ. ID. NO. 48), and MBP peptide(FFKNIVTPRTPPP (SEQ. ID. NO. 37), to the 5' end of the 25 amino acid flexible linker. ZC9479 (SEQ. ID. NO. 17) served as a 5' primer, overlapping the first 32 nucleotides of ZC9499 (SEQ. ID. NO. 23). The resulting 743 bp MBP- $\alpha 1\alpha 2$ IA^S PCR product contained, a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP (SEQ. ID. NO. 37), a 25 amino acid flexible linker (GGGGSGGGGSGGGGSGGGGSGGGGS SEQ. ID. NO. 32) attached to the N terminus of the IA^S $\alpha 1\alpha 2$ domain, which ended with a Spe I and Eco RI restriction site. The MHP- $\alpha 1\alpha 2$ fragment was restriction digested with Bam HI and Eco RI, and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ21 (MBP-$\alpha 1\alpha 2$ IA^S) (SEQ ID NOS:118 and 119) ~~SEQ. ID. NO. 52)~~.

Paragraph beginning at line 34 of page 71 has been amended as follows:

Four individual T cell lines derived from one HLA-DRB1*0404 patient (ThHo) were used to map the 74 synthetic GAD peptides, overlapping sets of 20 mers, that span the entire length of GAD 65 (SEQ ID NO:47) ~~(SEQ. ID. NO. 59)~~. Antigen presenting cells, BLS-DRB1*0404 and/or BLS-DRB1*0401 (Kovats et al, *J. Exp. Med.* 179:2017-22, 1994), were

loaded with peptide by incubating with peptide (about 50 mg/ml) for at least 5 hours. Reactivity of T-cells was determined as above. One peptide, hGAD 33 (PGGAISNMYAMMIARFKNFP SEQ. ID. NO. 40) stimulated 3 or the 4 lines with BLS-BI*0404. COOH terminal truncations of this peptide from 20 amino acids to an 11 amino acid fragment (PGGAISNMYAM SEQ. ID. NO. 39) when presented by either BLS-B1*0404 or BLS-DRBI*040l, stimulated only one or the T-cell lines. A 10 amino acid fragment (PGGAISNMYA SEQ. ID. NO. 41) stimulated the same T-cell line only when presented by BLS-BI*0404. This methodology quickly identifies peptide and HLA restriction of T-cell lines and clones as well as identifying GAD epitopes which stimulate T-cell lines derived from a prediabetic donor.

Paragraph beginning at line 20 of page 72 has been amended as follows:

Peptides amidated at the C terminus were synthesized by solid phase peptide synthesis (SPPS) using Fmoc chemistry. Chemicals used in the synthesis were obtained from Nova Biochem (La Jolla, CA) . The peptide was assembled on Rink amide MBHA resin (0.25 millimolar scale) starting from the C terminal end by using a 432A Applied Biosystems, Inc. (Foster City, CA) automated peptide synthesizer and solid phase strategy. The synthesis required double coupling to ensure completion of the coupling reaction, and HBtu-HOBt coupling chemistry was used. Bolded residues required at least double coupling (SRLSKVAPVIKARMMEYGTT-NH2) (SEQ ID NO:50) ~~(SEQ ID NO:59)~~. Each cycle included Fmoc deprotection of amine from the amino acid residue on the resin, and coupling of incoming Fmoc-amino acid. After successful assembly of the peptide, the resin was washed with dichloromethane and dried under vacuum for two hours. The peptide resin was resuspended in 10 ml trifluoroacetic acid (TFA) containing 1 ml of 4-methoxybenzenethiol and 0.7 g of 4-methylmercaptophenol as scavengers. This suspension was gently mixed at room temperature for 2 hours, then filtered through a PTFE filter, and the filtrate was collected in a capped glass bottle containing 1 liter organic solvent mixture (pentane:acetone = 4:1). The white precipitate was allowed to settle at room temperature for 1-2 hours, after which the crude precipitated peptide was isolated by centrifugation. The crude peptide was washed three times with the organic solvent mixture and dried under vacuum overnight.

Paragraph beginning at line 12 of page 76 has been amended as follows:

A series of C-terminal amidated GAD 65 (SEQ ID NO:50) ~~(SEQ. ID. NO. 59)~~ peptides were synthesized where one or more N-terminal or C-terminal amino acids were systematically truncated (Table 3).

Paragraph (Table 3) beginning at line 17 of page 76 has been amended as follows:

--Table 3 Truncated GAD65 peptides from amino acid 524 (1) to amino acid 543 (20). All peptides are amidated at the C-terminus.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SEQ ID NO:
S	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	50
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	51
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	52
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	53
				K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	54
					V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	55
						A	P	V	I	K	A	R	M	M	E	Y	G	T	T	56
							P	V	I	K	A	R	M	M	E	Y	G	T	T	57
								V	I	K	A	R	M	M	E	Y	G	T	T	58
									I	K	A	R	M	M	E	Y	G	T	T	59
										K	A	R	M	M	E	Y	G	T	T	60
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T		61
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G			62
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y				63
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E					64
	S	L	S	K	V	A	P	V	I	K	A	R	M	M						65
	S	L	S	K	V	A	P	V	I	K	A	R	M							66
	S	L	S	K	V	A	P	V	I	K	A	R								67
	S	L	S	K	V	A	P	V	I	K	A									68
	S	L	S	K	V	A	P	V	I	K										69
	S	L	S	K	V	A	P	V	I											70

Paragraph (Table 4) beginning at line 1 of page 78 has been amended as follows:

Table 4. Truncated GAD65 core pectides. The C-terminus of each peptide is amidated. 1 is amino acid 524, 20 is amino acid 543.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SEQ ID NO:
			S	K	V	A	P	V	I	K	A	R	M	M	E						71
				K	V	A	P	V	I	K	A	R	M	M	E						72
					V	A	P	V	I	K	A	R	M	M	E						73
						A	P	V	I	K	A	R	M	M	E						74
							P	V	I	K	A	R	M	M	E						75
			S	K	V	A	P	V	I	K	A	R	M	M							76
			S	K	V	A	P	V	I	K	A	R	M								77
			S	K	V	A	P	V	I	K	A	R									78
			S	K	V	A	P	V	I	K	A										79
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				80
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y					81
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E						82
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				83
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y					84
		L	S	K	V	A	P	V	I	K	A	R	M	M	E						85
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				86
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y					87
			S	K	V	A	P	V	I	K											88

Paragraph beginning at line 40 of page 85 has been amended as follows:

One hundred microliters of the cell-protease inhibitor mixture was added to each well of a 96-well round-bottom plate (Costar, Pleasanton, CA) . Fixed NOD cells were co-incubated with biotinylated, C-terminal amidated GAD65 peptide at a concentration of 10,000 nM and unlabeled, Ala scan peptides at concentrations of 100,000, 1,000 and 10 nM for 12-20 hours at 37°C. Mouse serum albumin (MSA) , a known allele-specific peptide (SEQ ID NO:89) ~~(SEQ. ID. NO. 61)~~ with high affinity for I-A^{g7}, was used as a positive control, and Eα, which binds to I-A^{d} but not to I-A^{g7}, served as a negative control (Reich et al., J. Immunol. 154: 2279-88, 1994) . Following incubation, the plates were vortexed and centrifuged in a Beckman GA-6R centrifuge for 10 minutes at 1500 rpm (Beckman, Fullerton, CA) . The supernatant was removed, and the cells were lysed in 60 µl/well of NP-40 lysis buffer (0.5% NP4O, 0.15 M NaCl, 50 mM Tris, pH 8.0, 0.01% sodium azide, and 1:50 dilutions of the protease inhibitor stocks D, E and F (Table 3). The cells were incubated on ice for 30 minutes, with mixing every 15 minutes, followed by centrifuging for 10 minutes at 1500 rpm to obtain a clear lysate.

Paragraph beginning at line 6 of page 91 has been amended as follows:

Newly diabetic NOD mice were irradiated (730 rad) and randomly divided into 4 treatment groups, and splenocytes were isolated as described above. Non-diabetic 7-8 week old, NOD recipient mice were divided into 4 groups. Group one received 1 x 10^7 splenocytes, injected intravenously. Six hours following the injection the mice received a second intravenous injection of either saline, 10 µg/mouse C-terminal amidated GAD65 peptide, or 10, 5, or 1 µg/mouse C-terminal amidated GAD65 peptide-MHC complex. Group two received 2 x 10^7 splenocytes, followed by injections with either saline, 10 µg/mouse C-terminal amidated GAD65 peptide-MHC complex, or 5 µg/mouse MSA-MHC complex. Group three received 1 x 10^7 splenocytes and injections of either saline, 10 µg/mouse C-terminal amidated GAD65 or 200 µg/mouse 10.2.16, an anti-class II antibody. Group four received 1 x 10^7 splenocytes followed by injection with either saline, 20 µg/mouse C-terminal amidated GAD65 peptide, or 1, 5 or 10 µg/mouse C-terminal amidated GAD65 peptide-MHC complex. Group four mice received only two treatments with peptide or peptide-MHC complex, one on day 0 and a second on day 4. All other groups received further treatments on days 8 and 12. The mice were tested for the onset of diabetes by urine analysis. On the day the first animal showed overt signs of diabetes, as

determined by urine and blood glucose levels, mice from each of the treatment groups were randomly selected, and urine and blood glucose levels determined for all selected mice, which were then sacrificed, and spleens and pancreases removed for immunohistochemical analysis. Saline-treated mice developed diabetes within about 12-20 days. Group one mice, which received four treatments of 10 μg peptide-MHC complex, had no significant development of disease by day 30, and did not develop disease until day 75. Those receiving 5 μg peptide-MHC complex had stabilized at 40% diseased mice by day 30, with a gradual increase in disease onset up to day 80, when there was 100% disease among the mice. Those mice in group four, which received only two treatments of peptide-MHC complex, experienced some delayed onset of disease, i.e., less than 50% of those mice receiving 10 μg of peptide-MHC had developed disease by day 30. Blocking with anti-MHC antibody in group three delayed the onset of disease, but provided less protection, i.e., over 75% of those mice receiving 10 μg peptide alone had developed disease by day 30. The C-terminal amidated GAD65 (SEQ ID NO:50) ~~(SEQ. ID. NO. 59)~~ peptide alone accelerated the onset of diabetes in this adoptive transfer model, while the peptide-MHC complex prevented onset of disease.